REMARKS

Applicant appreciates the thorough examination of the present application as evidenced by the Office Action dated April 22, 2004.

Claims 1 to 27 are under examination in this application. Claims 28 to 40 are withdrawn as directed to a non-elected invention. Claim 26 has been canceled herein without prejudice.

New claims 41 to 54 have been added to more fully define the invention, as discussed below.

Claims 1, 2, 5, 21, 23 and 27 have been amended for clarity to more particularly define the invention. Support for these amendments is found throughout the specification and in the original claim language as set forth below. No new matter is added by these amendments and their entry is respectfully requested. In light of the amendments presented herein and the following remarks, applicant respectfully requests entry of these amendments and allowance of the pending claims to issue.

I. REJECTION UNDER 35 U.S.C. § 112

The Examiner has rejected Claim 5 under 35 U.S.C. §112, second paragraph, as indefinite, on the grounds that the term "the filter" lacks antecedent basis. Claim 5 has been amended to recite that the solution is "passed through a filter", thus providing the required antecedent. Claim 5 has also been amended for clarification, to indicate that the filter is impregnated with the calcium-removing agent. Support for this amendment can be found at page 5, lines 30 to 32 of the specification.

Accordingly, Applicants respectfully request withdrawal of this rejection.

II. REJECTION UNDER 35 U.S.C. § 103

The Examiner has rejected Claims 1-7,10-17, 19, and 21-27 under 35 U.S.C. §103(a) as obvious over U.S. Patent No. 5,876,935 to Pankratz et al. (Pankratz et al.) in view of U.S. Patent No. 5,942,407 to Liotta et al. (Liotta et al.).

With respect to Claims 1, 2, 21, 23 and 25, the Examiner asserts that Pankratz et al. teach a method comprising the steps of combining with a sample a binding reagent labelled with a luminescent molecule that is capable of binding to an analyte, contacting the sample with another binding reagent that can be biotinylated, immobilized on a solid support such as super paramagnetic microspheres by means of avidin or streptavidin so that a complex with the analyte bound to the labelled binding reagent is formed, activating the luminescent label in the solid support-free sample or in the complex bound to the solid support, and determining the presence of analyte in the sample by detecting the light emitted from the activated luminescent label. The Examiner asserts that Pankratz et al. further teach that the label can be aequorin, activated by adding sufficient calcium ions.

The Examiner concedes that Pankratz et al. does not teach the presence of a calcium-caging compound in the support and does not teach the use of ultraviolet light to release calcium from the calcium-caging compound.

The Examiner relies on Liotta et al. as teaching the use of a calcium-caging compound immobilized in a support and use of ultraviolet light to activate the compound.

The Examiner then concludes that therefore it would have been obvious to include a calcium-caging compound immobilized in a support and ultraviolet light to activate the compound in the method of Pankratz et al., in order to extend the duration of light emission resulting from analyte detection.

Applicant respectfully submits that Pankratz et al. is directed to a chemiluminescent binding assay for measuring an analyte in whole blood without pre-treatment. The assay employs binding partners to the analyte, one of which is labelled with a luminescent molecule such as aequorin. The assay is carried out in solution, in a test tube or microtiter well (Column 5, lines 56 to 57). A calcium ion-containing solution is then added to the mixture to cause the aequorin to emit light,

which is detected by a luminescence detector and correlates with the amount of analyte present.

Liotta et al. is directed to a method for activating light emission by a photoprotein by providing a substrate having a zone coated or impregnated with either a dried salt of a metal cation or a dried caged metal cation compound to produce a dried metal cation zone, allowing reagent to contact the dried metal cation zone and releasing metal cation from the dried metal cation zone to activate the photoprotein labelled reagent.

In describing the use of a calcium-sensitive photoprotein, it is indicated that when the soluble photoprotein encounters the reporter system zone of the described device, "calcium ions are locally released from an immobilized state", i.e. released from the dried state by hydration of the dried reporter zone containing a calcium salt (Column 13, lines 25 to 28). This is the system used in the examples described in Liotta et al.

Liotta et al. then merely speculate that other techniques can be used to release calcium from the dried zone on hydration and proposes release of calcium from a caged calcium compound by introduction of ultraviolet light.

Liotta et al. do not consider any of the technical limitations and the problems to be overcome in supplying calcium in a caged form and releasing the calcium appropriately to activate a calcium sensitive chemiluminescent material.

More specifically, if caged calcium is used as calcium source, it is typically released by a pulse of ultraviolet light which can interfere with the measurement of the light emitted from the chemiluminescent material.

Liotta et al. do not teach or suggest how to provide for an emission-free time period before reliable quantifiable light emission from the chemiluminscent material.

Previous work in the field of fluorescent measuring systems have suggested the use of filters or shutters to separate light pulses, but this is a rather awkward or clumsy solution. Further, in fluorescent systems, the light pulse provided to stimulate fluorescence is usually of much less power than the pulse required to release calcium from a calcium-caging compound at a level sufficient to activate emission of light from a chemiluminescent protein.

It was not appreciated until the work of the present inventors that, as discussed at page 15 of the specification as filed, the luminescent material could be selected, in combination with the caged calcium compound, to provide a "dead time" or period of no light between pulsing the caged calcium with ultraviolet light and the initiation of light emission by the chemiluminescent as calcium source in this type of chemiluminescence-based assay.

The emission properties of the chemiluminescent material and the properties of the caged calcium are available in scientific publications so that one of ordinary skill in the art can, armed with the teachings of this application, select a calcium-sensitive chemiluminescent material, along with a calcium-caging compound, which provide a period of no light emission between the ultraviolet pulse and light emission by the chemiluminescent material.

In an effort to expedite prosecution, Claims 1, 21 and 23 have been amended to recite that the calcium-sensitive chemiluminescent material and the calcium-caging compound are selected so that there is a period with no light emission between the pulse of ultraviolet light effecting calcium release and the emission of luminescence by the luminescent material.

Applicant respectfully submits that Liotta et al. provide no teachings regarding how to stimulate sufficient light emission from the chemiluminescent protein using a caged calcium compound without premature triggering of light emission or how to avoid interference between the ultraviolet pulse needed to break the caged compound and the emitted light which has to be measured. Instead, Liotta et al.

provide mere speculation and provide no motivation to employ caged calcium compounds in the method of Pankratz et al.

Accordingly, Applicant respectfully submits that amended Claims 1, 21 and 23 are patentable over Pankratz et al. and Liotta et al. Moreover, Claim 2 is dependent from Claim 1 and Claim 25 is dependent upon Claim 21. It is respectfully submitted that Claims 1, 2, 21, 23 and 25, as amended, are not obvious over Pankratz et al. in view of Liotta et al.

With respect to Claims 3 and 14, the Examiner asserts that Pankratz et al. teach that the method is an immunoassay for detecting and quantifying an antigen. Since Claims 3 and 14 are dependent directly or indirectly upon amended Claim 1, it is respectfully submitted that these claims are patentable over Pankratz et al.

With respect to Claims 4, 5 and 6, the Examiner asserts that Liotta et al. teach the use of a calcium chelating agent such as EDTA or EGTA and that Pankratz et al. teach that the solution may be whole blood. As Claims 4, 5 and 6 are dependent directly or indirectly upon amended Claim 1, which as argued above is patentable over Pankratz et al. and Liotta et al., it is respectfully submitted that Claims 4, 5 and 6 are also patentable.

With respect to Claims 7 and 17, the Examiner asserts that Pankratz et al. teach that the calcium-sensitive luminescent material is aequorin. As Claims 7 and 17 are dependent directly or indirectly upon amended Claim 1, which as argued above is patentable over Pankratz et al., it is respectfully submitted that Claims 7 and 17 are also patentable over Pankratz et al.

With respect to Claim 10, the Examiner asserts that Liotta et al. teach that the substrate can be nitrocellulose. As Claim 10 is dependent upon amended Claim 1, which as argued above is patentable over Pankratz et al. and Liotta et al., it is respectfully submitted that Claim 10 is also patentable over these references.

With respect to Claim 11, the Examiner asserts that Liotta et al. teach that the substrate comprises a transverse stripe with immobilized second binding partner and a calcium caging compound. As Claim 10 is dependent indirectly upon amended Claim 1, which as argued above is patentable over Pankratz et al. and Liotta et al., it is respectfully submitted that Claim 11 is also patentable over these references.

With respect to Claim 12, the Examiner asserts that Liotta et al. teach that the calcium caging compound is loaded with an excess of calcium, in order to overcome any residual chelating agents. As Claim 12 is dependent upon amended Claim 1, which as argued above is patentable over Pankratz et al. and Liotta et al., it is respectfully submitted that Claim 11 is also patentable over these references.

With respect to Claims 15 and 16, the Examiner asserts that Liotta et al. teach that the binding assay can be an immunoassay or a nucleic acid hybridization assay. As Claims 15 and 16 are dependent upon amended Claim 1, which as argued above is patentable over the cited references, it is respectfully submitted that Claims 15 and 16 are also patentable over these references.

With respect to Claim 19, the Examiner asserts that Liotta et al. teach that the luminescence is measured by a photomultiplier. As Claim 19 is dependent upon amended Claim 1, which as argued above is patentable over the cited references, it is respectfully submitted that Claim 19 is also patentable over these references.

With respect to Claims 22 and 24, the Examiner asserts Pankratz et al. teach that all the components may be added at the same time. As Claims 22 and 24 are dependent upon amended Claims 21 and 23 respectively, and as amended Claims 21 and 23 are patentable over the cited references, as argued more fully above, it is respectfully submitted that Claims 22 and 24 are also patentable over these references.

With respect to Claim 26, the Examiner asserts Liotta et al. teach that the timing of the caged calcium can extend the length of the light pulse and furthermore

that Liotta et al. teach that the light detection is performed by utilizing a shutter assembly opened for a predetermined amount of time. Claim 26 has been canceled. It is, however, respectfully submitted that Liotta et al. do not teach time resolution of the UV pulse and the light emission from the chemiluminescent material as in amended Claims 1, 21 and 23, the light free time period being required to avoid premature emission from the chemiluminescent material interfering with reliable measurements. The shutter assembly referred to by Liotta et al. relate to an embodiment in which a photographic or photographic plate is used in a combination with the described assay system. This shutter assembly is not relevant to the basic method of stimulating light emission by the chemiluminescent material by added calcium. As discussed in detail above, Liotta et al. do not provide any teaching regarding how to avoid overlap between the UV pulse and the light emission by the chemiluminescent material or how to avoid premature triggering of light emission from the chemiluminescent material when using caged calcium compounds as a calcium source. Thus, it is respectfully submitted that the claims, as amended, are patentable over the cited references.

With respect to Claim 27, the Examiner asserts Liotta et al. teach the use of calcium chelating agents such as EDTA prior to the pulse of ultraviolet light. Liotta et al. do not teach that the solution prior to the addition of free calcium should contain less than 20 nM of calcium. Indeed, as discussed more fully below, their suggested use of calcium at levels of 10 mM to about 100 mM would have been expected, if caged calcium compounds were used, to produce a free calcium level considerably greater than 20 nM, as indicated in U.S. Patent No. US 5,446,186 to Ellis-Davies et al. (Ellis-Davies et al.). Furthermore, if excess EDTA is used, then the released calcium will bind to the EDTA, as indicated by Ellis-Davies, leaving little, if any, calcium to initiate emission from the chemiluminescent material.

Accordingly, it is respectfully submitted that Claims 1-7, 10-17, 19 and 21-27 are patentable over Pankratz et al. in view of Liotta et al.

Furthermore, Liotta et al. stipulate that the amount of calcium in the reporter system zone of the described device has to be high enough to overcome the presence of the chelating agent present to prevent premature excitation of the photoprotein by calcium in the sample. Liotta et al. describe a typical amount of Ca^{2+} in the reporter zone as from about 10mM to about 100mM. This is consistent with other previously described assay systems such as Pankratz et al., who employed a calcium concentration of 100mM.

Applicant respectfully submit that it is technically difficult to deliver calcium at a concentration of 10mM to 100mM in the reporter zone using a caged-calcium compound, while maintaining a pre-release level of free Ca^{2+} of less than 20 nM, which is required to prevent premature photoprotein excitation. This is confirmed by Ellis-Davies et al., cited by the Examiner, which shows, for example, that although the calcium-caging compound nitrophenyl-EGTA has a high affinity for calcium and could be used locally to deliver a high concentration of calcium, the level of free calcium before the ultraviolet pulse would be much higher than 20 nM, thereby triggering premature light emission.

Although Liotta et al. make a vague suggestion that caged calcium compounds could be used, they do not address this anomaly. In fact, it was assumed in the art that caged calcium compounds could not be used as the calcium source in assays based on calcium-sensitive luminescent materials for precisely this reason, that the pre-release calcium level could not be kept low enough to avoid premature triggering of light emission from the luminescent material. It is technically difficult to use a chelating agent such as EDTA to lower pre-release calcium levels while still being able to release enough calcium to trigger chemiluminescence, because the EDTA present at the time of calcium release would avidly take up released calcium.

Liotta et al. therefore **teach away** from the present invention by defining calcium levels thought not achievable using caging compounds without triggering

premature light emission. As described in the present application, at page 14, a pre-release calcium level of less than 20 nM is desirable. As seen in the examples, the calcium-caging compound is therefore preferably loaded to 75% or less with calcium.

In order to more fully describe the invention, new Claims 42 to 54 have been added. Claims 42 to 44 define the calcium concentration before the ultraviolet pulse as less than 20 nM. Support for these claims can be found at page 14, line 26 of the specification. Claims 46 to 48 define the loading of the calcium-caging compound as up to 75% calcium. Support for these claims is found at page 19 of the specification, in example 1. Claims 49 to 51 are directed to an embodiment of the invention employing aequorin as a luminescent material and DM-nitrophen as a calcium-caging compound. Support for these claims can be found, for example, in example 1, page 19. Claims 52 to 54 are directed to an embodiment employing obelin as luminescent material. Support for these claims can be found, for example, at page 3, line 24, of the specification.

The Examiner has further rejected Claims 8, 9, 13, 18, and 20 under 35 U.S.C. §103(a) as obvious over U.S. Patent No. 5,876,935 to Pankratz et al. (Pankratz et al.) in view of Liotta et al. and further in view of Ellis-Davies et al.

The Examiner asserts, with respect to Claim 13, that Pankratz et al. and Liotta et al. teach a method of a binding assay as previously discussed involving use of caged calcium compounds but do not disclose specific caged calcium compounds. Ellis-Davies et al. teach a number of calcium-caging compounds and teach that these compounds produce very high yields of liberated calcium ion. The Examiner asserts that it would, therefore, have been obvious to use DM-nitrophen or NP-EGTA as calcium-caging compounds in the method of Pankratz et al. and Liotta et al.

Firstly, as discussed more fully above, the teachings of Pankratz et al. and Liotta et al. cannot be combined to provide the method of amended Claims 1, 21 and 23. Furthermore, as also discussed fully above, the properties discussed in Ellis-

Davies et al. for the calcium-caging compounds and the level of calcium described as being required by Liotta et al., i.e. 10 mM to 100 mM, would lead one of ordinary skill in the art away from the claimed method, as one of ordinary skill in the art would assume that it was not possible to use such caged calcium compounds and avoid premature triggering of light emission by the chemiluminescent material. Accordingly, it is respectfully submitted that Claim 13, which is dependent upon amended Claim 1, is patentable over all of the cited references.

With respect to Claims 8, 9, 18 and 20, the Examiner asserts that Liotta et al. teach the use of ultraviolet light in the form of a light pulse to activate the caged calcium compound, that Ellis-Davies et al. specify the use of a laser at 347 nm and that Liotta et al. further teach that a photomultiplier is used to sense the luminescence. As Claims 8, 9, 18 and 20 are dependent directly or indirectly upon amended Claim 1, which as argued above is not rendered obvious by Pankratz et al., Liotta et al. and Ellis-Davies et al., Claims 8, 9, 18 and 20 which are dependent directly or indirectly upon amended Claim 1, are also non-obvious over all of the cited references.

For the reasons set forth above, Applicant respectfully submits that all of the pending rejections have been adequately addressed and that the present claims are in condition for allowance, which action is respectfully requested. The Examiner is

invited and encouraged to contact the undersigned directly if such contact will expedite the prosecution of this application to issue.

Respectfully submitted,

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